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A review on *in vitro* propagation of some medicinally important plant species of family Asparagaceae

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ABSTRACT
Plants belonging to family Asparagaceae are known to contain a number of compounds like phenolics, steroids, triterpenes, tannins, glycosides, alkaloids, 5-hydroxymethyl-2-furaldehyde, diosgenin, saponosides, lysine, serine, aspartic acid, threonine and many more. In addition to this, they are the sources of a number of valuable products like food and fodder, oils, drinks, cosmetics, fibres for making ropes and cords, drugs etc. It has been surveyed that due to increasing awareness about the valuable products obtained from them, they are depleting at an alarming rate, leading to their unscientific harvesting, overexploitation and increasing anthropogenic pressures like deforestation, habitat loss, expanding urbanization, excessive grazing, encroachment and bioprospecting for new sources. Since conventional means of propagation take long time for multiplication, plant tissue culture provides a tremendous potential solution for the propagation of these valuable plants ultimately leading to their conservation. This review emphasizes on the *in vitro* propagation protocols developed for many species of the family asparagaceae for their conservation and mass propagation by using different explants and nutrient media.
Key words: Asparagaceae; valuable products; plant tissue culture; explant, nutrient medium

INTRODUCTION

Asparagaceae is a family of flowering plants, placed under order Asparagales of monocots **[1].** The family consists of 7 sub families, 51 genera, 670 species **[2],** many of which has their own economic value and hence contribute to the floristic wealth of the world. Micropropagation technology, which is an alternative approach for plant propagation, has been exploited for number of plants **[3]**. Some medicinally important plant species of family Asparagaceae that have been successfully micropropagated at large scale are discussed below.

Genus *Asparagus*

It consists of medicinal shrubs valued for their medicinal properties. It includes about 300 species around the world. The roots of *Asparagus* are the main source of drug 'shatawar', the crude drug also used for secretion of milk and improving appetite in lactating women **[4].** The species of genus *Asparagus* for which successful micropropagation has been achieved are:

Asparagus racemosus* (L.)*: An *in vitro* propagation protocol was established for *A. racemosus*, (an important medicinal herb which contains asparagin **[5])** using shoot segments as explants**.** Callus was induced from shoot segments inoculated on MS medium supplemented with 2, 4-D and Kn. Regeneration of shoot buds and clonal multiplication of excised shoots was achieved on MS medium fortified with IAA and BAP. Rooting was achieved on half strength MS basal medium. The plants were successfully transfered to soil. Another protocol was optimised in which multiple shoots were initiated from shoot apex and nodal explants on MS medium supplemented with BAP (8.9 µM) and NAA (0.27 µM)**.** Elongation of shoots was achieved on MS medium supplemented with 15% coconut milk and 2-iP (19.6 µM). Rooting of *in vitro* raised shoots was achieved by a preculture treatment with MS medium supplemented with IBA (7.35 µM) for 48 hours and then transferred to MS medium supplemented with 15% coconut milk. Plantlets were successfully hardened and acclimatized after 5 weeks **[7].**Another *in vitro* protocol was standardized using nodal explants cultured on MS medium fortified with different combinations and concentrations of BAP and IAA Best results of shoot regeneration were obtained on MS medium supplemented with BAP (2 mg/l) and IAA (0.2 mg/l). Rooting of *in vitro* regenerated shoots was obtained on MS basal medium incubated in the dark **[8].**

***Asparagus officinalis* L.:** An efficient micropropagation protocol was developed for *A. Officinalis*, a medicinal plant used in treatment of

dropsy, schistosomiasis and tuberculosis, through *in vitro* shoot production on MS medium supplemented with NAA, Kn and BAP **[9]**,

***Asparagus Densiflorus* L.:** Successful reproducible clonal propagation protocol was developed for *A. densiflorus* using seeds as explant. Best response of seed germination was achieved by cold and wet seed stratification prior to culture **[10]**

***Asparagus adscendens* Roxb. :** *A. Adscendens* an important medicinal herb used in traditional medicinal remedies for treatment of spermatorrhoea, chronic leucorrhoea, diarrhoea, dysentery, asthma and fatigue **[11].** Successful propagation protocol was established for *in vitro* propagation of this plant species. A very high rate of multiple shoot production was achieved from nodal explants cultured on MS medium supplemented with NAA (0.27 µM), Kn (0.46 µM). Good rooting response was observed on MS medium supplemented with IBA (1.48 µM). After hardening plantlets were successfully transferred to soil with a high rate of survival **[12]**.

***Asparagus maritimus* L. Miller:** An efficient *in vitro* propagation protocol was developed for *A. maritimus* -A rare mediterranean salt-resistant species using excised explants from spear segments and cultured on MS (1962) medium supplemented with various growth regulators**.** Best shoot initiation was achieved on MS medium containing BAP (0.88 µM), Kn (0.93 µM), NAA (1.07 µM) and ancymidol (3.90 µM) and best shoot multiplication on MS medium supplemented with NAA (1.07 µM), Kn (0.93 µM), BAP (0.44 µM) and low concentrations of ancymidol. Shoot regenerated roots (82.0% rooting) on medium supplemented with NAA (1.07 µM) and higher concentrations of ancymidol **[13]**.

***Asparagus cooperi* Baker:** An *in vitro* propagation protocol was optimised for *A. cooperi* by using shoot tips and nodes as explants. Maximum number (62–65) of shoots was obtained from shoot tip explants cultured on MS medium containing BAP (2.0 mg dm^{-3}), adenine (80 mg dm^{-3}) and NAA (0.02 mg dm^{-3}) after 60 days of culture. Higher number of shoots was regenerated from nodal cultures after 30 days. Best rooting of *in vitro* shoots was achieved in potassium salt of IBA [14].

Genus *Agave*

The genus *Agave* contains 155 species (and over 200 varieties); 75 % of which are native to Mexico**.** *Agave* has been a renewable source of food, beverages (tequila), fibers (sisal), silage for livestock, drugs (saponins, sterols, steroidal alkaloids, and alkaloidalamines), ornamental plants (due to their distinctive leaf form and colour) and their useful products **[15]**. The species of genus *Agave* that have been micropropagated successfully are:

***Agave tequilana* F.A.C. Weber.:** An *in vitro* regeneration protocol was developed for *A. tequilana* by indirect organogenesis. Leaf segments and meristematic tissue were used as explants. Maximum callus proliferation was obtained on MS medium supplemented with NAA. Shoot regeneration was achieved from meristematic segments and callus on MS medium supplemented with 2, 4-D (1.1 µM) and BAP (4 µM). Shoots rooted in a hormone-free MS medium and acclimatized in a greenhouse with a 100% survival **[16]**.

Successful micropropagation protocol was standardised for three different varieties of *Agave* (*A. cantala* Roxb., A. *fourcroydes* Lem. and *A. sisalana* Perrine) using stolon planlets as explants and cultured on MS basal medium. The addition of combination of growth substances-NAA (0.075 mg/l), IBA (0.1 mg/l), Kn (0.5 mg/l) caused an extensive proliferation of multiple shoot primordia. Subculture of these on the same medium was successful for the multiplication. Shoots were rooted on hormone free MS medium and then transferred into a sand bed for acclimation before field planting **[17]**.

***Agave fourcroydes* Lem. :** An *in vitro* propagation protocol was developed for *A. fourcroydes.* It was observed that the NO_3^- NH_4^+ balance in the culture medium were key factors for controlling callus growth and organogenesis in rhizome cultures. Stem callus showed limited organogenic capacity, but high cytokinin concentrations induced adventitious shoot formation on stem explants. When these shoots were excised and sub cultured, new callus formed at their base from which new shoots arose. The shoots from stem explants and rhizome callus formed extensive root systems *in vitro* and were

transferred to pot culture with a 90% survival rate **[18]**.

Agave arizonica **Gentry &Weber.** : An efficient plant regeneration protocol was established from bulbil explants of *Agave arizonica*, an extremely rare and endangered plant species native to Arizona. Adventitious shoots were raised from callus grown on MS medium supplemented with 2,4-D (1.4 µM).These shoots were proliferated by subculture in media supplemented with combination of BAP (44.4 µM) and NAA (0.5 or 5.4 µM). Rooting was achieved on MS basal medium and plants were transferred to potting soil **[19]**.

Genus *Bowiea*

Bowies volubilis **Harv. *ex* Hook. F.:** An efficient *in vitro* propagation protocol was developed for *B. volubilis.* Callus was obtained from inflorescence segments on MS medium supplemented with 2, 4-D (1 mg/l) and 15% v/v coconut milk. Shoot primordia developed after 2–3 subcultures when auxin concentration was lowered. Rooted bulbous plants were obtained on MS basal medium. Direct shoot regeneration was also achieved from scales of regenerated bulbs used as secondary explants, on MS medium supplemented with BAP (2 mg/l) and 2, 4-D (0.05 mg/l). These shoots multiplied rapidly in shake cultures of liquid MS medium. Eighty percent of plants survived in potted soil **[20]**. Another micropropagation technique was devised for *B. volubilis* by culturing inflorescence stem on MS basal medium supplemented with 2, 4-D (1 mg/l) and BAP (1 mg/l)**.** Bulblet development, shoot elongation and rooting occurred on MS basal medium. Successful hardening was achieved in relatively low humidity **[21]**.

Genus *Beaucarnea*

Beaucarnea gracilis **and** ***Beaucarnea recurvata:*** An efficient protocol was developed for micropropagation of *B. gracilis* and *B. Recurvata,* two endangered and endemic Mexican species *In vitro* seedlings were used as explants and highest number of shoots per explant were obtained on MS medium supplemented with BAP (22.2 µm). Rooting of regenerated shoots was readily achieved on MS basal medium supplemented with activated charcoal. The rooted plants were successfully transfered to green house **[22]**.

Genus *Chlorophytum*

Chlorophytum borivilianum **Sant. et Fern:** *C. Borivilianum* is an endangered herb having number of medicinal properties like antidiabetic, antistress, anti-inflammatory, antioxidant, antimicrobial and aphrodisiac [**23, 24]**. An i*n vitro* clonal propagation protocol was developed for this plant species by culturing young shoot bases were cultured on MS medium supplemented with BAP (22.2 µM). The shoots multiplied at a rate of four-fold every third week. Rooting was achieved on 3/4-strength MS medium supplemented with BAP (9.8 µM). 67% of the micropropagated plants were successfully established in pots **[25].** Another i*n vitro* micropropagation protocol was developed for *C. Borivilianum* from four different explants viz., leaf base, shoot base, stem disc and tuber explants. Among these four explants stem disc and shoot base showed better response on MS medium supplemented with BAP (2 mg/l). Maximum number of leaves, shoot and shoot length was obtained on MS medium supplemented with BAP (2 mg/l) and root proliferation was obtained on MS medium supplemented with IBA (2-3 mg/l) and BAP (2 mg/l). *In vitro* rooted shoots were transferred and established under net house condition **[26]**. Another *in vitro* propagation protocol was developed for *C. borivilianum* and the effect of different PGRs, gelling agents, sucrose and heat shock on shoot multiplication and growth, rooting and survival of *C. borivilianum* was studied. Best shoot multiplication was obtained on MS basal medium containing BAP and 116–290 µM Sucrose. Rooting of *in vitro* raised shoots was achieved on basal medium as well as medium supplemented with IBA (5.0 µM). Among gelling agents, phytagel was found to be more effective for shoot proliferation. Successfully hardened plants showed 95% survival rate in soil containing Vermicompost **[27].** Another clonal propagation protocol was developed for *C. borivilianum***.** 90% shoot multiplication was obtained on MS medium supplemented with BAP (4.4 µM). Rooting of *in vitro* shoots was obtained on ½ MS medium supplemented with IBA (2.4 µM). *In vitro* raised plants were successfully acclimatised **[28]**.

Genus *Cordyline*

An efficient micropropagation protocol was developed for *Cordyline* to bypass the slow rate of multiplication of traditional methods. Stem cuttings

containing single nodes and shoot tips were used as explants. Best shoot regeneration was obtained on MS medium supplemented with BAP (4 mg/l), NAA (0.5 mg/ l) and GA_3 (5 mg/l). 85% *ex vitro* rooting was obtained in different hardening media **[29].**

Cordyline terminalis **(L) Kunth. :** Rapid multiplication of *C. terminalis* was achieved from shoot apex explant on MS medium supplemented with 3% (w/v) sucrose and different concentration and combinations of growth regulators**.** Shoot initiation and multiplication was obtained on MS medium supplemented with BAP in combination with adenine sulphate and IAA. Best shoot elongations was observed on ½ strength MS basal medium **[30]**. *In vitro* shoots were rooted on ½ MS medium supplemented with IBA. Rooted plants passed through a hardening phase prior to *ex vitro* transfer. Another *in vitro* propagation protocol was developed for *C. terminalis* by using stem explants. Induction and proliferation of shoots was obtained from stem explants inoculated on modified MS medium supplemented with BAP (0.5 ppm) **[31]**.

Genus *Drimia*

Drimia indica **(Roxb)***.* syn. ***Scilla indica*** **(Roxb):** Successful micropropagation protocol was developed for *Scilla indica* (Roxb**).** In this protocol somatic embryogenesis was induced from anther calli of *S. indica* on MS medium supplemented with NAA and coconut milk. Development of embryos was achieved on hormone-free MS medium **[32]**.

Drimia robusta **Baker:** An efficient *in vitro* plant regeneration protocol was developed for *D. Robusta.* Different developmental stages of somatic embryos, club-shaped embryos, cotyledon initiation, plumule initiation and plantlets were obtained directly from leaf explants cultured on MS medium containing 3.5 % (w/v) sucrose and different PGRs. Higher number of SEs and plantlets was obtained on MS medium containing sucrose (3.5 % (w/v)), picloram (10 µM), TDZ (1 µM) and glutamine (20 µM). The shoots were established on ½ MS medium followed by successful acclimatization in the greenhouse. Liquid somatic embryo medium (SEML) containing 500 mg of friable embryogenic callus produced different stages of SEs. Highest number of plantlets (9.0 ± 0.70) was obtained in SEML containing picloram (0.5 µM), TDZ (1 µM) and haemoglobin (25 mg/l). 90 % of SEs germinated on ½ strength MS medium supplemented with NAA (0.5 µM). All plantlets were successfully acclimatized in the greenhouse **[33]**.

Genus *Dracaena*

Dracaena fragrans **Ker. :** An *in vitro* propagation protocol was established for green-foliaged *D. Fragrans* using stem as explant. Callus was obtained from young stem segments cultured on MS medium supplemented with 2, 4-D (0.25–0.5 mg/l). Shoots were differentiated on MS medium fortified with BAP (0.5–1.0 mg/l) and IBA (0.5–2.0 mg/l) or NAA (0.1–0.2 mg/l). He found same medium suitable for shoot multiplication. Rooting was achieved on MS Medium supplemented with IBA (0.5 mg/l) and rooted plantlets were successfully hardened **[34]**.

Pleomele reflexa **Lam. cv. *Variegata***: Successful micropropagation protocol was standardized for *P. reflexa.* Seven to eight buds were induced from nodal explant cultured on ½ strength MS medium supplemented with BAP (0.05 mg/l) and NAA (2.0 mg/l). Shoot bud elongation as well as rooting was achieved on MS medium containing BAP (0.5 mg/l). However, root proliferation was obtained on MS basal medium. Plantlets were successfully transferred to small earthen pots after a short period of acclimatization **[35]**.

Dracaena marginata **cv. *Tricolour*.** Successful micropropagation protocol was standardized for *D. marginata* by using shoot-tips as explant and culturing them on MS medium supplemented with different combinations of BAP and IBA. Largest numbers of shoots per explant was achieved on MS medium supplemented with BAP (4.0 mg/l). The highest percentage of root formation (80%) from *in vitro* shoots was obtained on NAA (2 mg/l). The rooted plantlets were successfully acclimatized using a mixture of vermiculite and peatmoss in 1:1 ratio **[36].**

Dracaena surculosa **Lindl.** : Successful protocol was developed for regenerating *D. surculosa* through *in vitro* indirect shoot organogenesis using bud, leaf and stem explants cultured on MS basal medium. 63.2% callus was induced from stem explants and 69.6% from bud explants on MS medium supplemented with 2iP (49.2 µM) and IAA

(2.3 µM). 65.7% shoot regeneration was obtained from stem-derived callus on MS medium containing 2iP (61.5 µM) and IAA (1.1 µM) and 88% from bud-derived callus on MS medium supplemented with 2iP (49.2 µM) and IAA (1.1 µM). *In vitro* rooting was obtained on MS medium supplemented with IAA (2.0 µM). The plantlets after transplantation into a soilless substrate grew vigorously in a shaded greenhouse **[37]**.

Genus *Eucomis*

***Eucomis* L'Hér. :** An *in vitro* propagation protocol was developed for genus *Eucomis* L'Hér**.** Multiple shoot production was initiated in all species from leaf explants cultured on MS medium solidified with 2g ampel/l Gelrite®. The optimal hormone combination for shoot initiation in majority of the species was NAA (1 mg ampel/l) and BAP (1mg ampel/l). Rooting was obtained on MS medium fortified with IAA (1mg ampel/l), IBA (1mg ampel/l), or NAA (1mg ampel/l), depending on species. Acclimatized plantlets were potted in sand: soil mixture (1:1) **[38]**.

***Eucomis comosa* (Houtt.):** A highly efficient plant regeneration protocol was developed for *E. Comosa,* in which somatic embryos regenerated directly from the cut surface of *in vitro* leaves within 30 days. The well-developed plantlets were directly transplanted to peat moss: perlite: vermiculite (1:1:1) ratio on which 100% survival rate was obtained after 25 days of acclimatization **[39]**.

Genus Hosta

***Hosta sieboldiana* (Hook) Engl.**: An i*n vitro* propagation protocol was standardized for *H. Sieboldiana* by inoculating ovaries from immature florets on modified MS medium supplemented with thiamine (0.4 mg/l) and glycine (2.0 mg/l). Calli were produced on modified MS medium containing NAA (5.4 µM) and BAP (4.4 µM) which initiated shoots on MS medium containing BAP (0.4 µM). *In vitro* regenerated shoots rooted on MS medium supplemented with $AdSO_4$ (80 mg/l). Rooted plantlets were established in sterilized vermiculite containing MS mineral salts **[40]**.

Genus *Hesperaloe*

Hesperaloe parviflora (Torr.) J.M.coult.: Shoot cultures of *H. parviflora* were induced from pedicel bud explants of mature plant on a modified MS medium containing Nitsch vitamins and zeatin (1 µM). Best axillary shoot multiplication was obtained by adding BAP (6 µM) to the medium. Growing shoots on a medium supplemented with BAP (6 µM) for 6 weeks and then transferring cultures to BAP (1 µM) increased the number of transferable shoots. Shoots rooted easily *in vitro* or *ex vitro* and were easily acclimatized **[41]**.

Genus *Liriope*

***Liriope platyphylla* Wang et Tang:** *L. platyphylla,* a medicinal plant traditionally been used as a tonic, as an antitussive agent, and as an expectorant **[42]**.High frequency somatic embryogenesis and plant regeneration protocol was developed for this plant species. Embryogenic callus was obtained from zygotic embryos at a frequency of 33% on MS medium supplemented with 2, 4-D (4.52 µM). Upon transfer to ½ strength MS basal medium, the embryogenic calli gave rise to numerous somatic embryos, which then developed into plantlets. Regenerated plantlets were successfully transferred to potting soil **[43]**. Another protocol for this plant species was developed using meristems as explant. Shoot regeneration was obtained by culturing meristem explants on MS medium fortified with different concentrations and combination of cytokinins and auxins. Among the cytokinins BAP and Phenyl urea showed the highest efficiency for shoot initiation, whereas kinetin and zeatin showed higher efficiency for shoot growth. Among cytokinin/auxins combinations, zeatin with IAA (0.1 mg/l) proved optimal for yielding the highest number of shoots (4.2 per explant) **[44]**.

***Liriope spicata* (Thunb.) Lour. :** An *in vitro* propagation protocol was developed for *L. spicata* by using young leaves as explant. MS medium supplemented with BAP (0.4 mg/l) in combination with NAA (1.0 mg/l) and 2, 4-D (1.5 mg/l) proved to be ideal culture medium for callus induction. ½ strength MS medium supplemented with $AgNO_3$ (1.2 mg/l), BAP (0.5 mg/l) and NAA (0.1 mg/l) was the ideal culture medium for the callus and adventitious bud differentiation. Rooting of adventitious buds was achieved on ½ strength MS medium supplemented with IAA (0.2 mg/l) and NAA (0.1 mg/l) **[45]**.

Genus *Muscari*

***Muscari armeniacum* Leichtlin**: An efficient *in vitro* regeneration protocol was developed for *M. Armeniacum.* The highest number of somatic embryo formations was observed on LS (Linsmaier and Skoog) medium containing BAP (2.0 mg/l) and NAA (0.5 mg/l) with a mean of 7.9 somatic embryos per explant after 10 weeks of culture. Green nodular callus was also obtained on LS medium supplemented with BAP (5.0 mg/l) alone or in combination with NAA (0.5 mg/l). After transferring it on LS medium supplemented with GA_3 (0.5 mg/l) for 8 weeks, producing 23.3 mean number of immature bulblet. Immature bulblets produced *in vitro* were either embedded in a sodium alginate matrix for the encapsulation process or transferred directly to LS medium supplemented with or without GA_3 (0.5 or 1.0 mg/l) for growth and development for 6 weeks. Encapsulated bulblets were then stored at 4^0C in darkness for 10 weeks and almost all encapsulated bulblets retained their viability and resumed their growth under greenhouse conditions **[46]**.

***Muscari neglectum* Guss. Ex Ten.** : An *in vitro* plant regeneration protocol was developed for *M. neglectum* from protoplast culture which was initiated from regenerable embryogenic calli obtained from bulb culture on MS basal medium. Protoplast was isolated directly from embryogenic calli, embedded in Ca-alginate beads and cultured with nurse cells on MS medium supplemented with NAA and BAP (1 mg/l each), ascorbic acid (100 mg/l) and mannitol (0.5 M) at 25^0C in darkness. After 4 weeks of culture, microcalli appeared on the surface of the Ca-alginate beads. Beads transfered into MS medium supplemented with BAP (0.1 mg/l) increased the growth of embryogenic calli. Somatic embryo development was observed either on ½ strength MS basal medium or supplemented with AB (1 mg/l) under continuous illumination with fluorescent light. Maturated embryos germinated and then converted to plantlets after 3 months on ½ strength MS medium containing BAP (1 mg/l). The plantlets left in the medium produced bulbs after 5 months **[47]**.

***Muscari muscarimi* Medikus:** An efficient *in vitro* propagation protocol for *M. muscarimi* was developed by using twin bulb scale as explant. Maximum number of daughter axillary bulblets (19) and daughter adventitious bulblets (16) per twin bulb scale explant were regenerated on MS medium supplemented with different combinations and concentration of BAP (17.76 µM), NAA (10.74 µM) and BAP (17.76 µM), NAA (2.685 µM) respectively. The daughter bulblets regenerated on twin bulb scales could be easily rooted on MS medium containing IBA (4.9 µM). The daughter bulblets regenerated on MS medium containing BAP (17.76 µM) plus NAA (10.74 µM) were transferred to MS medium containing 30 g/l sucrose to break negative carry over effect of the dose of BAP and NAA, where they grew 2–3 roots of variable length. Daughter bulblet diameter was increased by culturing them on MS medium containing BAP (4.44 µM) and NAA (5.37 µM). The results verified that both age and the source of explants had significant effect on regeneration. In another set of experiments, twin scales were obtained from *in vitro* regenerated daughter bulblets, although they induced bulblets, yet their bulblet regeneration percentage, mean number of bulblets per explant and their diameter were significantly reduced. *In vitro* regenerated bulblets were acclimatized in growth chamber on peat moss, where they flowered **[48]**.

Genus Ophiopogon

***Ophiopogon japonicus* (L.f.) Ker Gawl**: An effective *in vitro* propagation protocol was devised for *O. japonicus* by growing meristems on MS basal medium that contained NAA or NAA and BAP. A larger proportion of meristems developed into plantlets or produced callus when transfered on MS basal medium. Callus grown in liquid culture without PGR'S produced single cells and cell aggregates. Larger cell aggregates formed embryo-like structures that produced roots, cotyledons, and then plantlets following transfer to solid medium **[49]**.

Genus *Polygonatum*

According to earlier classification systems, genus *Polygonatum* was included under family Liliaceae **[50]** but as per the revised APG III system (2009), it was shifted to family Asparagaceae.

***Polygonatum odoratum* (Mill) Druce. :** Successful micropropagation protocol was optimised for *P. odoratum* by using rhizome as explant. Rhizome explants were cultured on Anderson's basal

medium supplemented with different combinations of plant growth regulators. Adventitious shoots were induced directly on the surface of explants on medium supplemented with cytokinin alone **[51]**. Another successful *in vitro* propagation protocol was developed for *P. odoratum* by using rhizome as explant. Induction of callus and buds was achieved by culturing rhizome explants on MS medium supplemented with BAP and NAA. Rooting was obtained on half strength MS medium supplemented with NAA **[52].**

***Polygonatum verticillatum* (L.) All.** Successful micropropagation protocol was standardized for *P. Verticillatum* by culturing stem disc explants on MS medium fortified with different concentrations (0.25-10 mg/l) and combinations of cytokinins (BAP, Kn, TDZ) along with different concentrations (0.5-1.0 mg/l) of auxins (NAA, IBA, IAA). MS medium containing BAP (3.0-8.0 mg/l) showed better response of shoot regeneration while as shoot length and number of leaves were comparatively more in MS medium supplemented with TDZ. Best rooting was achieved on ½ strength MS medium fortified with NAA (0.5 mg/l) **[53]**.

***Polygonatum cyrtonema* Hua. :** An efficient micropropagation protocol was optimised for *P. cyrtonema* from rhizome explants cultured on MS (1962) medium Callus induction and shoot differentiation was achieved on MS medium supplemented with 2,4-D and BAP **[54]**.

Genus *Polianthes*

***Polianthes tuberosa* L.:** *In vitro* propagation protocol was developed for *P. tuberosa*, a tuberose plant gaining importance in pharmaceutical and perfume industries because of their peculiar secondary metabolic reactions for the synthesis of various commercial valuable compounds. Rhizome explant was inoculated on MS medium supplemented with different concentrations of IAA and BAP and obtained best explant regeneration and shoot differentiation frequency (2.2±1.2 shoots/explant) on MS medium supplemented with BAP (1.5mg/l) **[55]**. Another *in vitro* protocol for this plant species was developed by using outer and inner scales of bulb as explants and cultured on MS medium fortified with different concentrations of BAP, NAA and TDZ. It was found that BAP (3.0 mg/l) was optimum for inducing shoot formation. Increased multiple shooting rate of regenerated shoots was obtained on MS medium supplemented with BAP (2.5 mg/l) in combination with NAA (0.5 mg/l) and Kn (0.1 mg/l). Highest rooting frequency was obtained on MS medium supplemented with NAA (1 mg/l). Well developed mericlones were successfully shifted into green house for acclimatization **[56]**. Micropropagation protocol was also developed for two varieties of *Polianthes tuberosa* viz. 'Phule Rajni' & 'Calcutta Double' by using bud as explant. In 'Phule rajni' they achieved maximum shoot proliferation on MS medium fortified with BAP (1.0 mg/l) and Kn (1.0 mg/l) while in 'Calcutta double' maximum shoot proliferation was achieved on BAP (2.5 mg/l) and Kn (2.5 mg/l). Rooting of *in vitro* shoots was achieved on MS medium supplemented with combination of NAA (0.5 mg/l) and IAA (0.5 mg/l) in 'Phule Rajni' and NAA (3.5 mg/l) and IAA (0.5 mg/l) in 'Calcutta Double' within 7 days. Plants were successfully transferred to pots for acclimatization **[57]**.

Genus *Ruscus*

***Ruscus aculeatus* L.**: An *in vitro* protocol was optimised for callus induction and indirect shoot regeneration of *R. Aculeatus* MS medium supplemented with zeatin (1.0mg/l) and NAA (0.3mg/l) proved best for callus induction. Best shoot regeneration was obtained from callus on MS medium supplemented with BAP (1.0mg/l), zeatin (0.5mg/l) and NAA (0.1mg/l). Rooting was obtained on half strength MS medium with 0.3% active carbon **[58]**.

***Ruscus hypoglossum* L.**: In this protocol effect of some natural media was observed on *in vitro* shootlet proliferation behaviour of *R. Hypoglossum* and *Aspidistra elatior* Blume. In *R. hypoglossum*, the maximum number of shootlets produced per explant was obtained on ½ strength MS medium, but the tallest shoot length and greatest number of leaves per shoot were found on full strength MS medium. In case of *A. elatior*, the greatest numbers of shoots per explant were obtained in full strength MS medium while the tallest shoots were produced on 100g/l of either chick pea or barley medium **[59]**. Another protocol was developed for *R. hypoglossum* by inducing callus cultures on TDZ containing medium leading to indirect shoot regeneration. Callus induction was obtained on 30

gm/l of sucrose which dropped down with the increase of sucrose concentration. Increasing sucrose concentration influenced positively shoot proliferation **[60]**.

Genus *Scilla L.*

***Scilla hyacinthiana* (Roth) Macbride:** Successful micropropagation protocol was established for *S. hyacinthiana* by using young leaf as explant. Best explant regeneration was achieved on MS (1962) basal medium and best shoot multiplication on MS medium supplemented with NAA (1 mg/l) and Kn at various concentrations (1-6, 8 and 10 mg/l). Rooting of *in vitro* regenerated shoots was obtained on liquid MS basal medium with 2% sucrose. Rooted plantlets were transplanted in a mixture of soil and cow dung in 2: 1 ratio **[61]**.

Genus *Sansevieria*

***Sansevieria cylindrica* Bojer ex Hook.:** An efficient *in vitro* regeneration protocol was developed for *S. cylindrica* using *in vitro* leaves and nodule cultures High frequency callus regeneration was achieved on MS medium supplemented with 2, 4-D and 2, 4, 5-T. Best results of shoot regeneration from callus was achieved on MS medium supplemented with combination of BAP (5 µM) and NAA (2 µM) with maximum number 17.6±0.14 of shoots per culture. Best results of shoot regeneration from nodule culture were achieved on MS medium supplemented with BAP (5 µM) and NAA (1 µM) with maximum number 25±0.16 of shoots per culture. Among nodule culture, 2, 4-D generated nodules were more proliferative and regenerative. Rooting of *in vitro* shoots was achieved on ½ strength MS medium containing IBA (5 µM) with an average root number 3.5±0.18 and root length 6.5±0.14 cm **[62]**.

Genus *Veltheimia*

***Veltheimia bracteata* Bake.**: Micropropagation protocol was optimised for *in vitro* regeneration of *V. bracteata* through scape sections and floral part explants. Bulblets were regenerated from scape sections and bracts on MS medium supplemented with NAA and BAP and from these proliferating cultures were established **[63]**.

***Veltheimia bracteata* Bak. and *Veltheimia capensis* (L.) DC**. An *in vitro* propagation protocol was established for *V. Bracteata* 'Lemon Flame', *V. bracteata* 'Rosalba' and *V. Capensis.* In *V. bracteata* 'Lemon Flame' and *V. bracteata* 'Rosalba' shoot initiation and multiplication were obtained on MS medium supplemented with BAP (8.87 µM) and NAA (0.54 µM) from immature flower bud and leaf explants while in *V. capensis* shoot initiation and multiplication were obtained on MS medium supplemented with BAP (8.87 µM) and NAA (0.54 µM) from leaf explants. Maximum Rooting of *in vitro* regenerated shoots were obtained on MS medium fortified with NAA (4.46 µM). Rooted shoots were successfully acclimatized for 3 to 4 weeks. Overall survival percentage was 69% for *V. bracteata* 'Lemon Flame', 65% for *V. bracteata* 'Rosalba', and 83% for *V. Capensis* **[64]**.

Genus *Yucca*

***Yucca aloifolia* L.:** *Y. aloifolia* is a medicinal plant having oxytoxic and anti-inflammatory properties **[65].** A rapid *in vitro* propagation protocol was developed for its mass multiplication. *In vitro* shoot tips were cultured on half strength MS medium augumented with different combinations and concentration of BAP, NAA and TDZ. The highest number of shoots (6.6) was obtained on medium supplemented with TDZ (4.5 µM) and NAA (1.1 µM). Rooting was achieved on ½ or ¼ strength MS basal medium and medium supplemented with IBA (2.5 or 4.9 µM) and charcoal. The rooted plantlets were successfully acclimatized in soil **[66].** An *in vitro* seed germination protocol was also developed for this species. 100% *in vitro* seed germination was achieved within 154 days in hormone-free Monnier medium. Adventitous shoots were obtained on Quorin and Lepover medium supplemented with BAP and NAA from epicotyl explant. The best result for rooting was achieved on MS medium supplemented with IBA (1mg/l) **[67]**.

DISCUSSION:

From the above observations it is clear that different efficient micropropagation protocols have been successfully standardized for different species of family Asparagaceae. Many explants of different plant species of this family have been used among which stem, leaf, nodal and rhizome explants showed the best response. In *Asparagus densiflorus, Beaucarnea gracilis, Beaucarnea recurvata, Sansevieria cylindrica, Yucca aloifolia* secondary explants have also been used. In most of the cases, MS medium supplemented with different

concentrations of auxins (mostly NAA) and cytokinins (mostly BAP) either alone or in combinations was used for shoot regeneration. However, in *Drimia robusta, Polygonatum verticillatum, Polianthes tuberose, Ruscus hypoglossum, Yucca aloifolia* kinetin, zeatin and TDZ proved best to induce shoots. For rooting, mostly MS basal medium or MS medium supplemented with IBA proved to be effective. Activated charcoal was also used for rooting of *Ruscus hypoglossum* **[59]** and *Yucca aloifolia* **[66].** *Ex vitro* rooting was achieved in *Cordyline* **[29].** In addition to MS medium some plants were propagated by using other growth media like Nitsch (N6) medium as *in Hesperaloe parviflora* **[41],** Linsmair-Skoog medium in *Muscari armeniacum* **[46],** Anderson's basal medium in *Polygonatum odoratum* **[51],** Chick pea medium and barley medium in *Aspidistra elatior* **[59].** Seed germination of *Yucca aloifolia* was achieved in hormone-free Monnier medium and adventitious shoots on Quorin and Lepover medium **[67].** Growth adjuvants like glutamine, AgNO3 (for callus proliferation of *Liriope spicata* **[45],** 2-ip for callus induction of *Dracaena surculosa* **[37]** proved very effective. Somatic embryogenesis was induced in *Scilla indica* using coconut water as a growth adjuvant **[32].** In most if the protocols agar was used as gelling agent but in some cases like in *Chlorophytum borivilianum* phytagel was used as gelling agent **[27]**. In *Eucomis* gelrite was used as gelling agent **[38].**

CONCLUSION

The documentation of above comprehensive review related with micropropagation of some species of family asparagaceae will help in sustainable development of these economically important plants as many plant species of this family have been exploited from their natural habitat which has rendered them threatened. A number of threatened species of this family like *Polygonatum verticillatum, Asparagus racemosus, Beaucarnea gracilis, Beaucarnea recurvata, Agave arizonica, Chlorophytum borivillianum* have been conserved by using *in vitro* propagation methods. Keeping in view the above *in vitro* standardized protocols, it can be concluded that these techniques have not only helped in mass propagation of these plants but also in conservation for sustainable development. From the above review, it can also be concluded that among different Nutrient Mediums available, MS Medium is the best for micropagating different plant species of family Asparagaceae. From the comprehensive literature survey, among different growth regulators, BAP individually and in combination with NAA is suitable for shoot regeneration IBA for root regeneration.

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